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White Paper

Optimization of Transient Protein Expression in CHO Cells

Lonza's new GS Discovery® process combines the advantages of a transient approach with those of stable pools

Peter O'Callaghan, Head of Expression System Sciences, Biologics and Licensing, Lonza

Recombinant therapeutic protein expression using mammalian platforms can be achieved using a range of workflows optimized for different objectives. These processes include transient transfection, and the creation of stable pools and clonal cell lines. Transient and stable pool processes are generally used for discovery through early-stage development to enable rapid production and screening of large numbers of protein candidates, while stable clonal cell lines are used for GMP clinical and commercial manufacturing. In this white paper, we describe how Lonza provides a set of optimised transient expression tools to support early stage expression of a range of therapeutic proteins.

Introduction

Transient transfection is a relatively low-cost, rapid method for producing recombinant proteins, but generally provides fairly low titers as expression is predominately episomal and no selection for plasmid transfection is applied. By contrast, stable pools are generated following transfection by applying selection pressure so that only cells with integrated DNA can grow. The selection process adds significant time to the development timeline, but higher titers can be achieved (Table 1).

Table 1
Features of transient transfection and stable pool generation

Process	Relative performance attributes: Speed	Cost	Titer
Transient transfection	● Fast	● Low	● Low
Stable pool generation	● Slow	● High	● High

Regardless of the method, to triage therapeutic candidates early in development, recombinant proteins produced during discovery and early-phase development must have product quality attributes (PQAs), such as isoform composition, percent aggregates and post-translational modifications, broadly comparable to material produced using a stable cell line. This is a particular concern when using alternative cell lines such as HEK for transient expression before moving to a Chinese hamster ovary (CHO) host for stable expression.

Typically, stable CHO cell pools created using transposase systems such as GS piggyBac® support significantly higher titers than those obtained from transient transfections. They also have the additional advantage of supporting cryopreservation, thereby enabling future rounds of product generation upon revival. However, the longer timeline associated with generating stable pools means that transient expression will usually be selected when speed is of the essence. To close the titer gap with stable pools and more fully realize the speed advantage of transient expression, optimization of the transient expression workflow is required.

An ideal solution would offer speed and also afford high titers of recombinant proteins with PQAs comparable to those generated using stable processes (we deem these PQAs 'CHO-like'). At Lonza we have been optimizing the stable pool expression format in recent years, particularly since the introduction of the GS piggyBac® hyperactive transposase technology. We therefore sought to apply the learnings from that work to create an optimized transient expression process that merges the best attributes of both traditional transient and stable pool expression.

The new GS Discovery® solution from Lonza, part of the GS Gene Expression System®, meets this need by offering such a hybrid process. It is to our knowledge the first example wherein a transposase enzyme has been used to boost titers from a transient transfection process over such a short timeframe. The GS Discovery® solution also includes a standard transient process for applications where speed and low cost are most important. Both leverage the high-performing GS Xceed® CHOK1SV GS-KO® cells.

Transient transfection vs. stable pool generation

Prior to transient transfection, a DNA plasmid that encodes a therapeutic protein is generated (vector). The vector is transfected into the cell using a chemical transfection reagent such as polyethylenimine (PEI), or by a physical method such as electroporation. A sufficient percentage of the transfected cells are assumed to have taken up the vector, and no selection pressure is applied, even though a percentage of the cell population will likely not contain the vector (Figure 1). The transfected cells are grown for a limited period of time. During this time, cells that have taken up the vector express the therapeutic protein, which is typically harvested after approximately two weeks. Longer growth periods are generally not recommended because the non-integrated vector is lost from the cells over time.

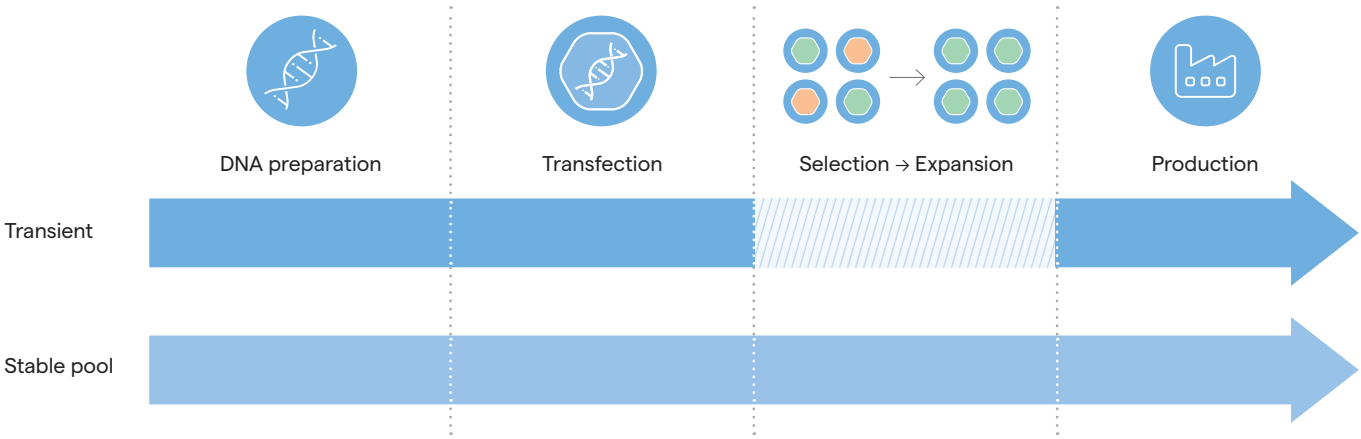

Figure 1
Typical processes for transient transfection and stable pool generation

When generating a stable pool, the same vector is used, although the cells may be transfected with the vector using a different method to a transient transfection process. The key difference between the transient and stable pool workflows, however, is the introduction of a discrete selection step to ensure that only cells that have the vector stably integrated into their genomes are expanded (Figure 1). Typically, a chemical reagent that stops the growth of cells that do not contain the vector is applied. Lonza's GS Xceed® CHOK1SV GS-KO® cell line, for instance, uses the selective agent methionine sulfoximine (MSX) in combination with the removal of L-glutamine from the growth medium.

After selection, the remaining cells containing the integrated DNA are expanded to generate the stable pool. At this point, media and feeds are applied and process conditions are adjusted to ramp up protein expression via a dedicated production phase, which can be performed for a longer period of time than for transient expression, as the integrated vector is not lost over time.

The selection and expansion phase can take between two to three weeks, including time for recovery after chemical treatment and two or three additional passages.

Transient transfection is therefore faster and less costly than stable pool generation because less time and fewer resources are required, but because the expression vector is not integrated into the genome, protein yield is lower. With stable pool generation, the addition of a selective pressure increases the proportion of cells expressing the protein product, and also means that more protein may be expressed per cell. To achieve this elevated titer, increased time and overall resource input is required compared to a traditional transient process.

Harnessing the power of GS piggyBac® to boost transient titers

Efforts to optimize CHO transient transfection titers have focused on the transfection process itself (e.g. electroporation vs. chemical methods), the cell culture media, use of chemical enhancers (e.g. sodium butyrate, valproic acid), expression vector design (e.g. EBNA-1/OriP system) and the use of modified process conditions (e.g. low temperature). However, due to the specific (genetic, epigenetic) nature of each CHO host cell line, the overall transient transfection workflow may need to be re-optimized for each host, with unpredictable results. Almost inevitably, therefore, the resultant transient process differs substantially from the processes involved in creating stable pools and clones, further complicating product expression across the development cycle.

Ideally, to simplify workflows and to reduce the risk of process-related impacts on product quality, a common set of tools would be used for both transient and stable expression. In the new GS Discovery® GS piggyBac® transient process, we take a technology (transposases) normally applied to stable transfection workflows and apply it to a transient process. Using this approach, the cell line, expression vectors and integration technology are consistent across the different expression formats from transient to stable pools and on to stable clones. This offers one system that can support projects from discovery phase through to commercial production.

Lonza's GS Discovery® solution offers two transient processes to suit customer requirements. The first, the GS piggyBac® transient process, is a novel hybrid approach to transient transfection that combines elements of traditional transient transfection with elements of stable pool generation. Good titers and CHO-like PQAs are obtained with the greater speed and lower cost associated with transient transfection — by combining the selection, expansion and production phases of stable pool generation into one integrated step (Figure 2 – next page). The second is the standard GS® transient transfection process, which doesn't utilize GS piggyBac®, and is suitable for applications where speed and cost are the primary considerations rather than overall harvest titer.

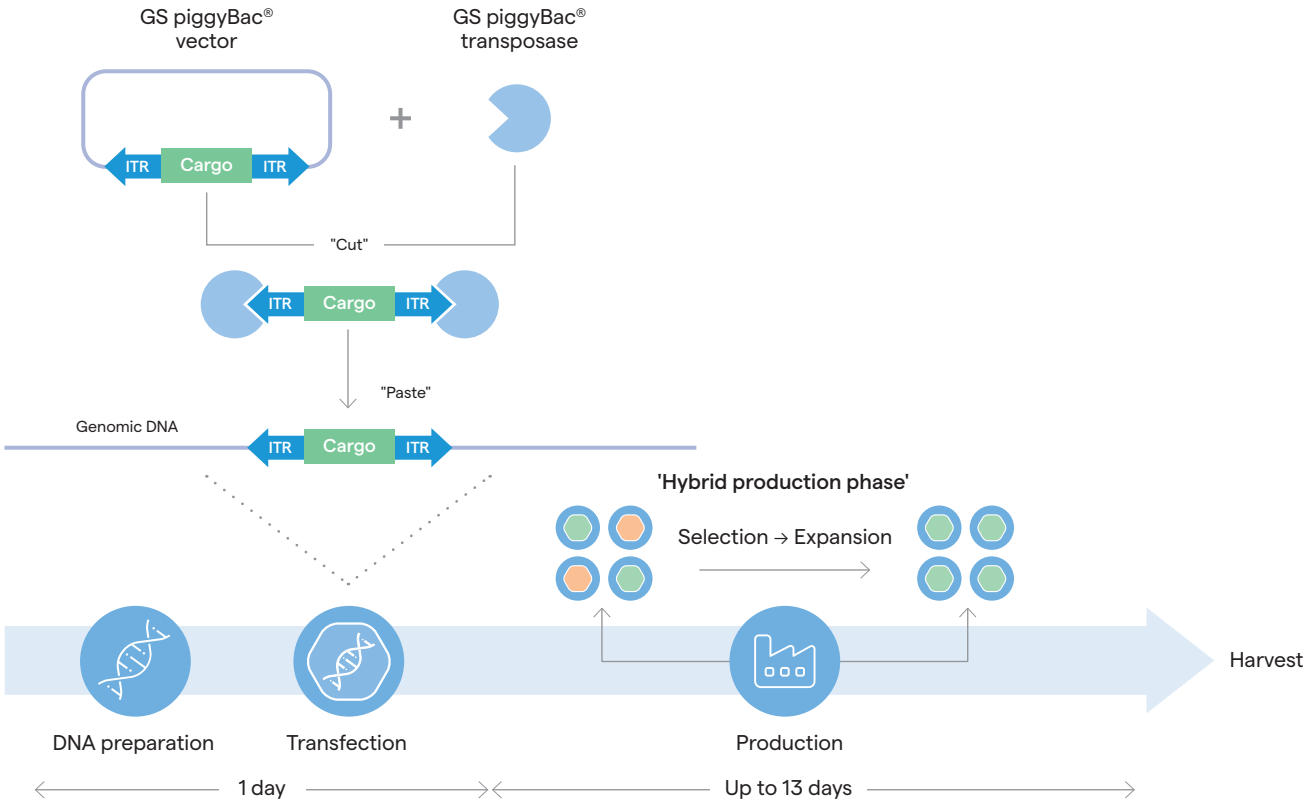

Figure 2
GS Discovery® GS piggyBac® transient process

In the GS Discovery® GS piggyBac® transient process, Lonza's GS piggyBac® transposase technology is leveraged to insert the DNA into the cell genome, preferentially targeting stable regions of the genome associated with highly expressed genes (Figure 2).

More specifically, the GS piggyBac® system uses an engineered, hyperactive transposase enzyme in combination with inverted terminal repeats (ITRs) contained within the GS piggyBac® compatible GSquad® integration vector encoding the therapeutic protein. Cells transfected in this manner exhibit improved gene expression efficiency, even for large DNA cargos, which are often associated with more complex and challenging protein formats.

The GS piggyBac® transient process harnesses the efficiency of the GS piggyBac® transposon technology to proceed straight from transfection to protein production without a separate selection phase. A mild selection process during the combined 'selection, expansion and production phase' is achieved by withholding glutamine from the media. This approach is effective because the integration vector encodes the glutamine synthase gene, and the high integration efficiency of the GS piggyBac® technology means there is sufficient production of glutamine by the cells.

Selection, expansion and production are thus combined into a hybrid, single phase to support high titers across a range of formats. As is the case with traditional transfection, harvest is completed 14 days after initiation.

Substantial titer increases

Exploiting the GS piggyBac® transposase technology in transient expression provides substantial increases in titer compared to the values obtained using Lonza's legacy process that used a simple electroporation method to deliver the expression vector (Figure 3). Up to approximately a 30-fold increase in titer was observed for a range of different molecular formats including monoclonal antibodies (mAbs) and bispecific antibodies (bsAbs). The average harvest titer for these runs was 336 mg/L.

Also shown in Figure 3 are results obtained using a commercially available CHO transient transfection kit using a lipid-based transfection method. The GS piggyBac® transient process provides comparable or higher titers across a range of product types, showing its broad utility for recombinant protein expression.

When the GS piggyBac® transient IgG1 (mAb 1) material was measured for key PQAs, including the major N-glycans, the aggregate percentage, and profiling for the main isoforms, we observed 'CHO-like' PQAs – similar to those of proteins stably expressed from GS Xceed® CHOK1SV GS-KO® derived clones generated using the same expression vectors and GS piggyBac® transposase technology (Figure 4). This highlights the benefits of harmonizing the core technology across expression protocols.

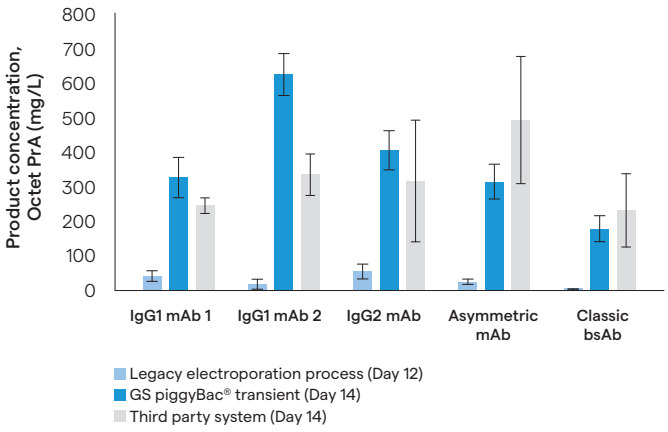

Figure 3
Expression titers obtained using the GS piggyBac® transient process, Lonza's legacy electroporation process and a commercially available third-party system.

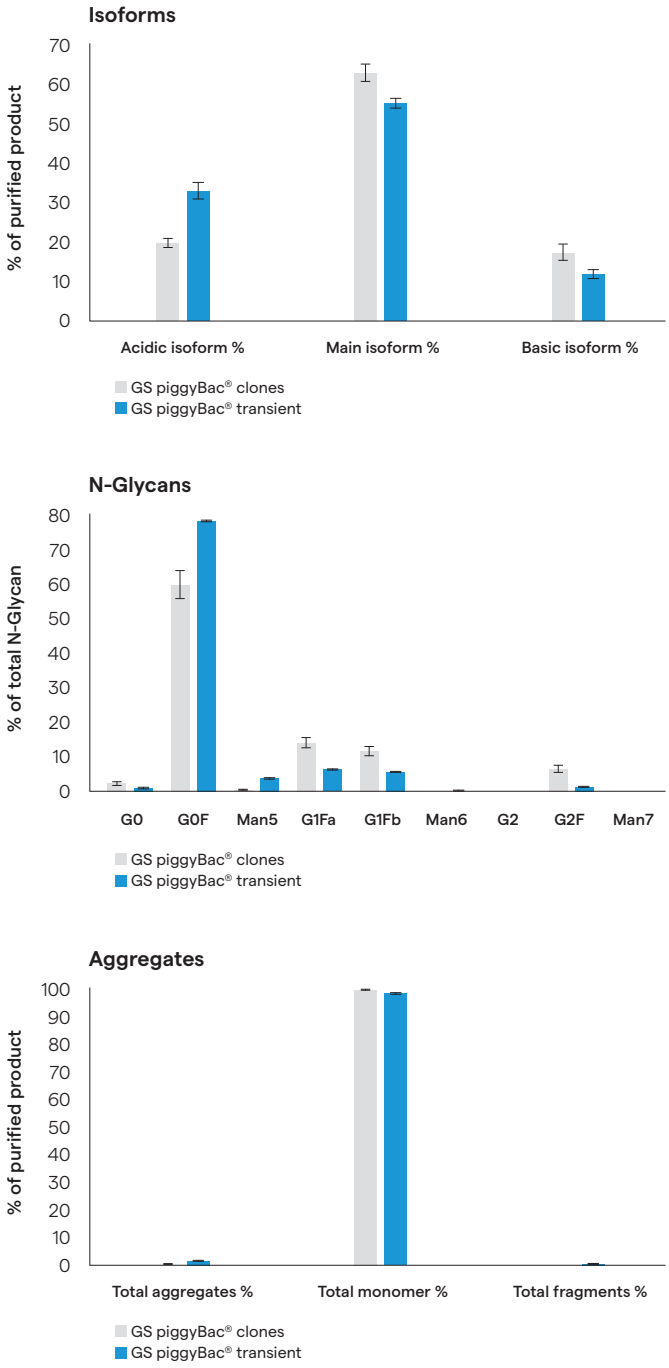

Figure 4
Comparison of PQAs of an IgG1 mAb produced using the GS Discovery® GS piggyBac® transient process and a cohort of clonal cell lines.

Flexible protein expression options with GS Discovery®

Requirements for therapeutic protein product expression, as outlined in the introduction, differ at early and late development stages. During discovery and preclinical phases, the emphasis is typically on speed, and the lower titers offered by traditional transient processes may be sufficient to meet material needs. For the most promising candidates, however, often more extensive testing is performed and thus greater quantities of product are required. For these cases, speed is still highly desirable, but often must be sacrificed in order to generate stable pools with the necessary productivity.

The GS Discovery® GS piggyBac® transient process helps to address the latter challenge, offering higher titers in a modified transient process that takes much less time than stable pool generation. However, in addition, the GS Discovery® system also offers a standard GS® transient process that does not utilize GS piggyBac®. It is a PEI-based transient protocol that provides speed coupled with lower resource requirements, which also delivers material with CHO-like product quality.

As can be seen in Figure 5, product titers generated from the GS piggyBac® transient process and standard GS® transient process are comparable up to approximately days 8 – 10 for an IgG1 mAb, IgG2 mAb and a bsAb generated using Lonza's bYlok® technology [1]. Beyond day 10, however, the GS piggyBac® transient process drives significantly higher titers. The increase is particularly dramatic for the complex bsAb antibody format.

Thus, developers of recombinant proteins can choose which GS Discovery® expression strategy is most appropriate for their specific projects and end goals. The standard GS® transient process offers material generation as quickly as possible using minimal resources. The GS piggyBac® transient process provides much higher titers in exchange for an extended culture time for those that require higher product titers.

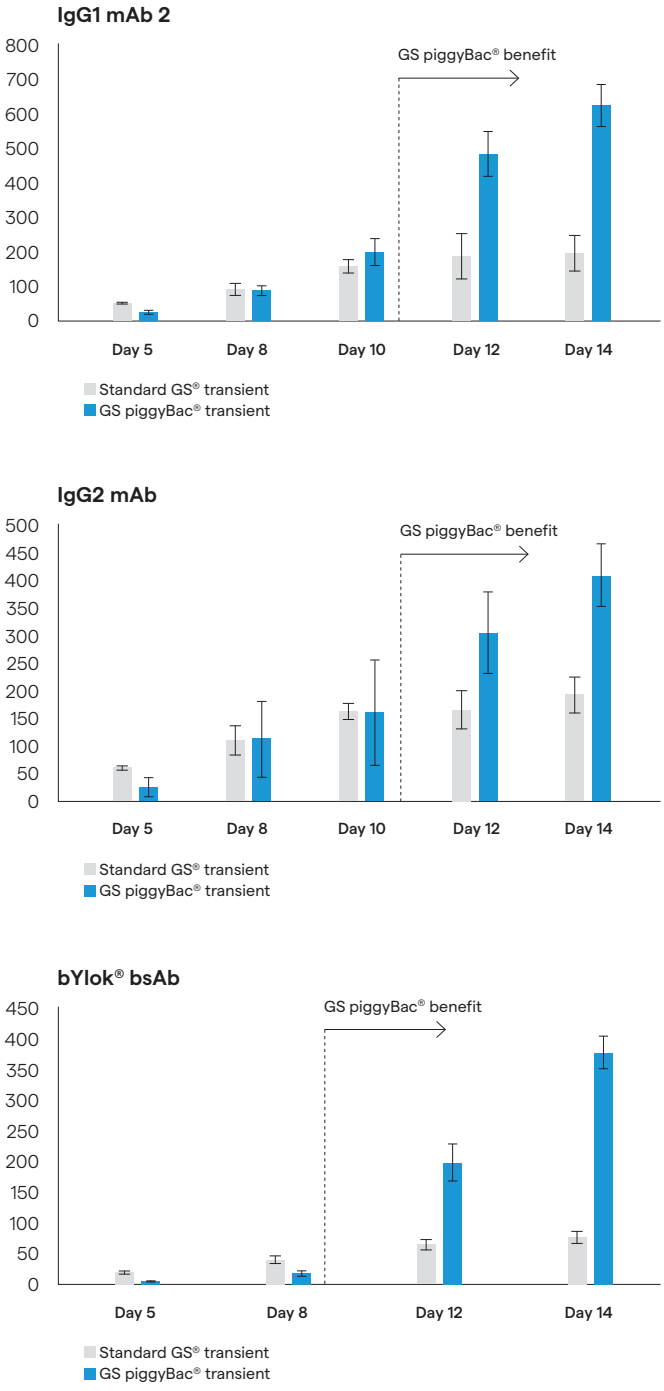

Figure 5
Expression results for Lonza's GS Discovery® GS piggyBac® transient and GS® transient processes

Conclusions

Access to different methods for protein expression facilitate the development of recombinant protein therapeutics. Lonza offers an optimized set of expression tools to support the production of proteins with high titers across the development spectrum from discovery through GMP manufacturing.

The new GS Discovery® transient processes enable protein production at high, competitive titers for transient systems. The proteins produced using these technologies exhibit robust, CHO-like quality attributes. Transition from transient to stable production is facilitated through the use of the same parental cell line and vectors from early phase to commercial from a single supplier. Most importantly, customers can choose which protocol works best for their projects depending on their need for speed or higher titers.

The optimized GS Discovery® transient expression processes are offered as part of Lonza's comprehensive, integrated and proven GS Gene Expression System®. The system includes the GS Xceed® CHOK1SV GS-KO® cell line, GSquad® vectors and GS piggyBac® technology, in addition to the GSv9® media and feed system and extensive know-how provided through the GS® Manual portal exclusively to licensees.

References

[1] bYlok® Bispecific Pairing Technology | Antibody Design | Lonza

Learn more about
Lonza's expression technologies

Contact us
Email: **licensing@lonza.com**
Web: **www.lonza.com/biologics/expression-technologies**

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